IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:	Confirmation No. 9701
BRENNEMAN et al.	Examiner: Standley, Steven H.
Application No.: 10/049,587	Technology Center/Art Unit: 1649
Filed: February 12, 2002	DECLARATION OF DR. ILLANA GOZES UNDER 37 C.F.R. §1.132
For: ORALLY ACTIVE PEPTIDES THAT PREVENT CELL DAMAGE AND DEATH	
Customer No.: 45115	

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Illana Gozes, Ph.D., being duly warned that willful false statements and the like are punishable by fine or imprisonment or both (18 U.S.C. § 1001), and may jeopardize the validity of the patent application or any patent issuing thereon, state and declare as follows:

1. All statements herein made of my own knowledge are true, and statements made on information or belief are believed to be true and correct.

2. I am currently a Professor of clinical biochemistry at Tel Aviv University I am Director of the Adams Super Center for Brain Studies & Edersheim Levi-Gitter fMRI Institute, also at Tel Aviv University. I have been the incumbent of the Lily and Avraham Gildor Chair for the Investigation of Growth Factors at Tel Aviv University, since 1997. I am also the Chief Scientific Office and Director at Allon Therapeutics, Inc. in Vancouver, Canada.

I received a Ph.D. from The Weizmann Institute of Science in 1979, and was a Haim Weizmann Postdoctoral Fellow at the Massachusetts Institute of Technology from 1979-1980. I was a Research Associate and Visiting Scientist at the Salk Institute and the Scripps

Exhibit A

Clinic and Research Foundation from 1981-1982. I was a Senior Scientist/Associate Professor at The Weizmann Institute of Science from 1982-1989. I was a visiting scientist in developmental neurobiology at NICHD, NIH from 1989-1990. My affiliation with Tel Aviv University began in 1990. I was a Fogarty-Scholar-in-Residence at NIH from 1995-1996 and an adjunct scientist in developmental neurobiology at NIH from 2003-2004.

3. I have received a number of scientific awards and prizes, including the Juludan Prize and the Teva Founders Prize for exceptional scientific studies and the Bergmann Prize and the Neufeld award for outstanding/leading US-Israel BSF grant proposals. I am currently Editor-in-Chief of The Journal of Molecular Neuroscience and I currently sit on the editorial boards of the American Journal of Alzheimer's Disease, the International Journal of Peptide Research & Therapy and the journal Peptides. I am an author on more than 197 research papers and am an author or co-author of numerous reviews and book chapters. A copy of my curriculum vitae is attached hereto as Exhibit B and includes a list of selected publications.

4. The present invention is an all-D amino acid ADNF I active core site peptide, proteins and peptides comprising the all-D amino acid ADNF I active core site, and compositions comprising the all D-amino acid core site proteins and peptides. Also claimed are methods to reduce neuronal cell death using the all-D amino acid ADNF I active core site proteins, peptides and compositions.

5. I have read and am familiar with the contents of this patent application. In addition, I have read an Office Action, dated October 19, 2006, received in the present case. It is my understanding that the Examiner alleges that the claimed invention is obvious over Brenneman *et al.* (US Patent No. 6,174,862) in further view of Voet (1995) and Goodman (1986). The Office Action also cited Rozhavskaya-Arena *et al. Endocrinology* 141:2501-2507 (2000) as representative of the state of the art at the time of filing.

6. According to the Office Action, Brenneman *et al.* teaches the sequence of the ADNF I peptide, but does not teach incorporation of D amino acids into the peptide. Also according to the Office Action, Voet and Goodman teach that D-amino acids are more resistant

to proteases than L-amino acids and that D-amino acid peptides are particularly advantageous when administered to patients and, thus, those of skill would have had a reasonable expectation of success to make useful D-amino acid ADNF I peptides and to perform the claimed methods using D-amino acid peptides. Finally, the Office Action alleges that Rozhavskaya-Arena *et al.* show that testing of the activity of peptides containing D-amino acids was routine at the time of filing. This declaration provides evidence that the biological activity of the all D-amino acid ADNF I active core site peptide was unexpected.

7. When designing the all D-amino acid ADNF I and ADNF III peptides, I expected to use those peptides as negative controls, *i.e.*, peptides that do not have biological activity. This expectation was based on the understanding of D-amino acid peptide structure in the art at the time of filing. A reference that describes the prevailing view of D-amino acid structure is submitted as Exhibit D: Guptasarma, *FEBS* 310:205-210 (1992). Guptasarma discusses the structure of peptides with inverted chirality, reversed amino acid sequence, or both: normal all L-amino acids, normal all D-amino acids (inverso), retro all L-amino acids (retro, reversed amino acid sequence), and retro-all-D amino acids (retro-inverso, reversed amino acid sequence). To obtain a peptide with activity like the unmodified all L-amino acid peptide, the chirality and amino acid sequence must both be changed. ". . . [N]ormal-all-D and retro-all-L analogues generally do not possess biological activity. On the other hand retro-all-D analogues have been found to possess biological activity. . . " Guptasarma, page 205, citations omitted. Thus, Guptasarma teaches that an all-D-amino acid peptide with an unmodified amino acid sequence was not likely to have biological activity. The cited references provide no reason to expect success in synthesizing a biologically active all-D-amino acid peptide in view of the teachings of Guptasarma. Brenneman *et al.* does not disclose peptides that contain D amino acids. Voet *et al.* teach only that peptides containing D-amino acids are less susceptible to attack by proteases. Voet *et al.* provides no expectation of success in making a biologically active all-D-amino acid peptide. Goodman *et al.* teach addition of a polymer of amino acids to certain drugs, *e.g.*, non-amino acid small molecules. Only non-naturally occurring, synthetic peptides are disclosed to contain D-amino acids. No biological activity is associated with the synthetic peptides. Biological activity of the drug cogener is associated with the small molecule, not the D-amino acid peptide. Goodman *et al.* does not disclose attaching any all D-amino acid peptide

with a biological activity to the drug and provides no expectation of success in producing a biologically active all-D-amino acid peptide, with or without a drug.

8. Rozhavskaya-Arena *et al.* provides evidence that all D-amino acid peptides are useful as negative controls because, as predicted by the model of Guptasarma , all D-amino acid peptides do not have biological activity. Rozhavskaya-Arena *et al.* substituted a D-amino acid for an individual L amino acids in the leptin peptide. The unmodified leptin peptide is denoted OB3 in the reference. A negative control, an all D-amino acid leptin peptide, is denoted [D]-OB3. Administration of the unmodified OB3 peptide caused mice to decrease food intake and minimize weight gain as compared to control, untreated mice. The amount of food taken in and weight gain by mice administered the all D-amino acid [D]-OB3 peptide was similar to that of control, untreated mice. *See, e.g.*, Table 4, Figure 3A, and Table 5. For example, Table 4 shows that control, untreated mice and mice treated with [D]-OB3 were about 10% heavier at the end of the experimental period. In contrast, mice treated with active OB3 were only 0.8% heavier at the end of the experimental period. Thus, Rozhavskaya-Arena *et al.* demonstrate that, at the time of filing, biological activity associated with an all-D-amino acid peptide was unexpected.

9. In view of the foregoing, it is my scientific opinion that the biological activity of the all D-amino acid ADNF I peptide was unexpected and that the cited references do not provide any data or disclosure that would provide a reasonable expectation of success for making a biologically active all-D-amino acid peptide.

Date: March 19,2007 By: Illana Gozes

Illana Gozes, Ph.D.

BLK:blk
61004197 v1

EXHIBIT B

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Illana Gozes	Professor of Clinical Biochemistry, The Lily and Avraham Gildor Chair for the Investigation of Growth Factors, Director the Adams Super Center for Brain Studies, Tel Aviv University; Chief,Scientific Officer, Allon Therapeutics Inc.
eRA COMMONS USER NAME IGOZES@POST.TAU.AC.I	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Tel Aviv University (TAU)	B.Sc.	1973	Biology, Life Sciences
Feinberg Graduate School, Weizmann Institute	Ph.D.	1979	Neurobiology
Massachusetts Institute of Technology (MIT)		1979-1980	Neuroendocrinolgy
The Salk Institute & Scripps Clinic		1981-1982	Molecular Neurobiology

A. Positions and Honors.

Positions and Employment

1982-1987 Senior Scientist of Hormone Research, Weizmann Institute of Science
1987-1989 Associate Professor of Hormone Research, Weizmann Institute of Science
1989-1990 Visiting Scientist in Developmental Neurobiology, NICHD, NIH.
1990-1993 Associate Professor of Chemical Pathology, Tel Aviv University (TAU)
1993- Professor of Clinical Biochemistry, Tel Aviv University (TAU)
2001-2003 Chief Executive Scientist, Chair of the Board; Allon Therapeutics, Inc. La Jolla, CA, USA
2003-2004 Chief Scientific Officer, Vice Chair of the Board, Allon Therapeutics, interim CEO (summer2004)
2004- Chief Scientific Officer and Director; Allon Therapeutics, Inc. Vancouver, BC, Canada
2006- Director, Adams Super Center for Brain Studies & Edersheim Levi-Gitter fMRI Inst. At TAU

Other Experience and Professional Memberships

1988-1989 Visiting Scientist in Molecular Genetics, NICHD, NIH
1989-2003 Invited Guest Scientist in Developmental Neurobiology (Summers) NICHD, NIH
1993-1994 Chair, Department of Chemical Pathology, Tel Aviv University (TAU)
Chair, Forum of Preclinical Department Heads, Sackler School of Medicine, TAU
1994-1995 Chair, Department of Clinical Biochemistry, Tel Aviv University
1995-1996 Fogarty Scholar-in-Residence, NIH
1997- The Lily and Avraham Gildor Chair for the Investigation of Growth Factors, Tel Aviv University
1997- Senate committee for TAU Masters Degrees (to 2001), The Israeli Neuroscience Society (to 1999).
The Sackler Faculty of Medicine: R&D Committee. Graduate School Committee (-2001)
1998- The Sackler Faculty of Medicine: Head, International Advisory Committee (-2001)
Member, Committee of School Heads (-2000)
1998- Co-Editor-in-Chief, 2000- Editor-in-Chief, The Journal of Molecular Neuroscience, Humana Press
Member: the scientific advisory board: Institute for the study of aging (ISOA), NY, USA
1999-2003 Member of the Sackler Faculty Pre-Clinical Appointment and Promotion Committee
2000- Executive guest editor: Current pharmaceutical Design, Clore Scholars Board Member (-2003)
2001- Co-director, the TAU-Sackler Med. School-NIH student program in Women Health
Chair of the organizing committee Neuropeptides 2001 Meeting (Israel), Co-Chair, American Meeting
2003-2004 Adjunct Scientist in Developmental Neurobiology, NICHD, NIH
2002- TAU Senate Committee for postdoctoral fellows (-2003), Sackler Faculty of Medicine, Grants Committee (-2006); Co-chair: the American Neuropeptides Summer Meeting.
2003 Selection committee for Gotlieb Award (ISOA, NY, USA), VIP and Related Peptide, Scientific Advisory Committee; Secretary General the European Neuropeptide Club
2005- Editorial Board, American Journal of Alzheimer's Disease; International J of Peptide Res. & Therap.
International Advisory Committee, the VIP PACAP and Related Peptides Meeting
Member, Scientific Advisory Committee: The United State Israel Binational Science Foundation (-200
2006- Editorial Board, Peptides, member Professorial Chairs Committee, Sackler Faculty of Medicine,
Member, TAU Committee on Faculty Equal Rights, TAU Board of Governors

Honors (selected)

1977	Landau Prize (Miphal Ha-pais) Excellent Ph.D., Katzir Fellowship
1978-1980	Chaim Weizmann Post-Doctoral Fellowship, EMBO short-term fellowship (1978)
1982-1985	The Bergmann Memorial Fund Research Prize, for excellent BSF grant (nationwide)
1983-1989	The first Incumbent of Samuel O. Freedman Career Development Chair
1991	The Juludan Prize for outstanding research achievements
1993	Teva Founders Prize for opening new horizons in medical research in Israel
1994	Fogarty International Scholar
1997-	The Lily and Avraham Gildor Chair for the Investigation of Growth Factors
2000-2003	The Neufeld Grant Award for excellent BSF grant application in health sciences, nation-wide
2000	Best scientific work award: The Israeli Society for Laboratory Sciences
2003-2004	The Mariana and George Saya grant, HIV and Parkinson (Tel Aviv Univ).
2003/4	Best student best paper, Brain Research (Dr. Albert Pinhasov), Poster award – VIP-PACAP meeting (student, Inna Divinski); Dan David Scholarship Student (Shmuel Mandel, student)
2006	Boaz Moav Prize for Develop. Biology [Switzerland Inst at Tel Aviv Univ (S. Mandel, student)

B. Selected peer-reviewed publications (in chronological order). (from >197 reviewed publications)

1. I Gozes, H Schmitt, UZ Littauer Translation in vitro of rat brain messenger RNA coding for tubulin and actin. Proc Natl Acad Sci USA 1975; 72:701-705.
2. I Gozes, MD Walker, AM Kaye,UZ Littauer Synthesis of tubulin and actin by neuronal and glial nuclear preparations from developing rat brain. J Biol Chem 1977;252:1819-1825.
3. I Gozes, UZ Littauer Tubulin microheterogeneity increases with rat brain maturation. Nature 1978;276: 411-3
4. I Gozes, KJ Sweadner Multiple forms of tubulin are expressed by a single neuron. Nature 1981;294:477-480
5. I Gozes, CJ Barnstable Monoclonal antibodies that recognize discrete forms of tubulin. Proc Natl Acad Sci USA 1982; 79:2579-2583.
6. Y Gozes, MA Moskowitz, TB Strom, I Gozes Conditioned media from activated lymphocytes maintain sympathetic neurons in culture. Dev Brain Res 1983;6: 93-97.
7. M Bodner, M Fridkin, I Gozes VIP and PHM-27sequences are located on two adjacent exons in the human genome. Proc Natl Acad Sci USA 1985;82:3548-3551.
8. F Baldino, S Fitzpatrick-McElligott, I Gozes, JP Card Localization of VIP and PHI-27 messenger RNA in rat thalamic and cortical neurons. J Mol Neurosci 1989;1:199-207.
9. E Giladi, Y Shani, I Gozes The complete structure of the rat VIP-gene. Mol Brain Res 1990;7: 261-267.
10. I Gozes, SK McCune, L Jacobson, D Warren, TW Moody,M Fridkin, DE Brenneman An antagonist to vasoactive intestinal peptide: effects on cellular functions in the central nervous system. J Pharmacol Exp Therap 1991;257:959-966.
11. P Gressens, JM Hill, I Gozes, M Fridkin, DE Brenneman Growth factor function of vasoactive intestinal peptide in whole cultured mouse embryos. Nature 1993; 362:155-158.
12. I Gozes, J Glowa, DE Brenneman, SK McCune, E Lee, H Westphal Learning and sexual deficiencies in transgenic mice carrying a chimeric vasoactive intestinal peptide gene. J Mol Neurosci 1993; 4:185-193.
13. I Gozes, A Bardea, A Reshef, R Zamostiano, SZhukovsky, S Rubinraut, M Fridkin, DE Brenneman Novel Neuroprotective strategy for Alzheimer's disease: inhalation of a fatty neuropeptide. Proc Natl Acad Sci USA 1996;93:427-32.
14. DE Brenneman, I Gozes A femtomolar-acting neuroprotective peptide. J Clin Invest 1996;97: 2299-307.
15. M Bassan, R Zamostiano, A Davidson, A Pinhasov E Giladi, O Perl, H Bassan, C Blatt, G Gibney, G Glazner, DE Brenneman, I Gozes Complete cDNA sequence of a novel protein containing a femtomolar-activity-dependent neuroprotective peptide. J Neurochem 1999;72:1283-93.
16. I Gozes, O Perl, E Giladi, A Davidson, O Ashur-Fabian, S Rubinraut, M Fridkin Mapping the active site in vasoactive intestinal peptide to a core of four amino acids: neuroprotective drug design. Proc Natl Acad Sci USA 1999;96: 4143-48.
17. P Gressens, L Besse, P Robberecht, I Gozes, M Fridkin, P Evrard Neuroprotection of the developing brain by systemic administration of vasoactive intestinal peptide derivatives. JPharmacol Exp Ther1999;288:1207-13
18. D Offen, YG Sherki, E Melamed, M Fridkin, DE Brenneman, I Gozes Vasoactive intestinal peptide (VIP) protects from dopamine toxicity: relevance to Parkinson's disease. Brain Res. 2000; 854:257-62.
19. I Zemlyak, S Furman, DE Brenneman I Gozes A novel peptide NAP prevents death in enriched neuronal cultures. Regulatory Peptides 2000;96:39-43.

20. I Gozes, E Giladi, A Pinhasov, A Bardea, DE Brenneman Activity-dependent neurotrophic factor: intranasal administration of femtomolar-acting peptides improve performance in a watermaze. J Pharmacol ExpTherap 2000;293:1091-98.
21. O Blondel, C Collin, B McCarran, S Zhu, R Zamostiano, I Gozes, DE Brenneman, RDG McKay A Glia-derived Signal Regulating Neuronal Differentiation. J Neurosci 2000; 20:8012-20.
22. R A Steingart, B Solomon, D E Brenneman, M Fridkin, I Gozes VIP and peptides related to activity-dependent neurotrophic factor protect pc12 cells against oxidative stress. J Mol Neurosci. 2000;15:137-45
23. L Beni-Adani, I Gozes, Y Cohen, Y Assaf, RA Steingart, DE Brenneman, O Eizenberg, V Trembolver , E Shohami A peptide derived from activity-dependent neuroprotective protein (ADNP) ameliorates injury response in closed head injury mice. J Pharmacol Exp Therap 2001; 296:57-63.
24. R Zamostiano, A Pinhasov, E Gelber, R A Steingart, E Seroussi, E Giladi, M Bassan, Y Wollman, H J Eyre, JC Mulley, D E Brenneman and I Gozes Cloning and Characterization of the Human Activity-Dependent Neuroprotective Protein (ADNP). J Biol Chem 2001;276:708-14.
25. CY Spong, DT Abebe, I Gozes, DE Brenneman and JM Hill Prevention of fetal demise and growth restriction in a mouse model of fetal alcohol syndrome. J Pharmacol Exp Ther 2001; 297, 774-9.
26. O Ashur-Fabian, E Giladi, S Furman, RA Steingart, Y Wollman, M Fridkin, DE Brenneman, I Gozes Vasoactive intestinal peptide and related molecules induce nitrite accumulation in the extracellular milieu of rat cerebral cortical cultures. Neurosci Lett 2001; 307:167-70.
27. I Gozes Neuroprotective peptide drug delivery and development: potential new therapeutics. Trends in Neurosci. 2001;24:700-5.
28. J Romano, L Beni-Adani, OL Nissenbaum, DE Brenneman, E Shohami, I Gozes A single administration of the peptide NAP induces long-term protective changes against the consequences of head injury: gene Atlas array analysis. J Mol Neurosci 2002;18: 37-45.
29. RR Leker, A Teichner, R Nussen, N Grigoriadis, Y Cohen, H Ovadia, DE Brenemann, M Fridkin, E Giladi, J Romano, I Gozes NAP, a femtomolar-acting peptide, protects the brain against ischemic injury by reducing apoptotic death. Stroke 2002;33:1085-92.
30. R Zaltzman, SM Beni, E Giladi, A Pinhasov, RA Steingart, J Romano, E Shohami, I Gozes Injections of the neuroprotective peptide NAP to newborn mice attenuate head-injury related dysfunction in adults. Neuroreport 2003;14:481-4.
31. A Pinhasov, A,M Mandel, Torchinsky, E Giladi, Z Pittel, AM Goldsweig, SJ Servoss, DE Brenneman, I Gozes Activity-Dependent Neuroprotective Protein: a novel gene essential for brain formation. Brain Res Dev Brain Res 2003;144:83-90
32. O Ashur-Fabian, Y Segal-Ruder, E Skutelsky, RA Steingart, E Giladi, D E Brenneman, I Gozes The neuroprotective peptide NAP inhibits the aggregation of the beta-amyloid peptide. Peptides 2003;24: 1413-23
33. DE Brenneman, CY Spong, HM Hauser, D Abebe, A Pinhasov, T Golian, I Gozes Protective peptides that are orally active and mechanistically non-chiral. J Pharmacol Exp Ther 2004;309:1190-7.
34. RN Alcalay, E Giladi, CG Pick, I Gozes Intranasal administration of NAP, a neuroprotective peptide, decreases anxiety-like behavior in aging mice in the elevated plus maze. Neurosci Lett 2004;361:128-31.
35. R Zaltzman A Alexandrovich, SM Beni, V Trembovler, E Shohami, I Gozes Brain injury-dependent expression of activity-dependent neuroprotective protein. J Mol Neurosci 2004; 24:181-7.
36. I Divinski, L Mittelman, I Gozes A femtomolar-acting octapeptide interacts with tubulin and protects astrocytes against zinc intoxication J Biol Chem 2004;279:28531-8.
37. M Zusev, I Gozes Differential regulation of activity-dependent neuroprotective protein in rat astrocytes by VIP and PACAP. Regul Pept 2004;123:33-41.
38. S Furman, RA Steingart, S Mandel, JM Hauser, DE Brenneman, I Gozes. Subcellular localization and secretion of activity-dependent neuroprotective protein in astrocytes. Neuron Glia Biology 2004;1:193-9.
39. S Furman, JM Hill, I Vulih, R Zaltzman, JM Hauser, DE Brenneman, I Gozes Sexual dimorphism of activity-dependent neuroprotective protein in the mouse arcuate nucleus. Neurosci Lett 2005;373:73-8.
40. VL Smith-Swintosky, I Gozes, DE Brenneman, MR D'Andrea, CR Plata-Salaman. Activity-dependent neurotrophic factor-9 and NAP promote neurite outgrowth in rat hippocampal and cortical cultures. J Mol Neurosci. 2005;25:225-38.
41. WA Lagreze, A Pielen, R Steingart, G Schlunck, HD Hofmann, I Gozes, M Kirsch. The peptides ADNF-9 and NAP increase survival and neurite outgrowth of rat retinal ganglion cells in vitro. Invest Ophthalmol Vis Sci. 2005;46:933-43.

42. R Zaltzman, A Alexandrovich, V Trembovler, E Shohami, I Gozes. The influence of the peptide NAP on Mac-1-deficient mice following closed head injury. Peptides. 2005; 26:1520-7.
43. L Visochek, RA Steingart, I Vulih-Shultzman, R Klein, E Priel, I Gozes, M Cohen-Armon. PolyADP-ribosylation is involved in neurotrophic activity. J Neurosci. 2005;25:7420-8.
44. I Gozes, BH Morimoto, J Tiong, A Fox, K Sutherland, D Dangoor, M Holser-Cochav, K Vered, P Newton, PS Aisen, Y Matsuoka, CH Van Dyck, L Thal. NAP: research and development of a peptide derived from activity-dependent neuroprotective protein (ADNP). CNS Drug Rev. 2005;11(4):353-68.
45. M Holtser-Cochav, I Divinski, I Gozes. Tubulin is the target binding site for NAP-related peptides: ADNF-9, D-NAP, and D-SAL. J Mol Neurosci. 2006;28(3):303-7.
46. RA Steingart, I Gozes. Recombinant activity-dependent neuroprotective protein protects cells against oxidative stress. Mol Cell Endocrinol. 2006 Jun 27;252(1-2):148-53. Epub 2006 May 15.
47. M Rotstein, H Bassan, N Kariv, Z Speiser, S Harel, I Gozes. NAP enhances neurodevelopment of newborn apolipoprotein E deficient mice subjected to hypoxia. J Pharmacol Exp Ther. 2006 Jul 5.
48. I Gozes, I Spivak-Pohis. Neurotrophic Effects of the Peptide NAP: A Novel Neuroprotective Drug Candidate. Curr Alzheimer Res. 2006;3(3):197-9.
49. I Divinski, M Hotlser-Cochav, I Vulih-Schultzman, RA Steingart, I Gozes. Peptide Neuroprotection through specific interaction with brain tubulin. J. Neurochem. 2006; 98:973-84.
50. S. Mandel, G. Rechavi, I. Gozes. Activity-dependent neuroprotective protein (ADNP) differentially interacts with chromatin to regulate genes essential for embryogenesis. Develop Biol, 2006 Dec 1; [Epub]
51. Y Matsuoka,A Gray,C Hirata-Fukae, S Sakura Minami,E Graeme Waterhouse, MP Mattson, FM LaFerla, I Gozes, PS Aisen. Intranasal NAP administration reduces accumulation of amyloid peptide and tau hyperphosphorylation in a transgenic mouse model of AD at early pathological stage. J Mol Neurosci. In press.

Chapters in books + reviews (selected **last four years)**

1. I Gozes, S Furman, RA Steingart, A Pinhasov, I Vulih, J Romano, R Zaltzman, R Zamostiano, E Giladi, S Rubinraut, M Fridkin, J Hauser, DE Brenneman Femtomolar-acting neuroprotective peptides: application for inhibition of Alzheimer s disease in: drug discovery and development for Alzheimer's disease 2000 Springer Publishing Company, Proc. 1st ISOA investigators meeting (H Fillit, AO Connonel eds) 204-14, 2002
2. I Gozes, R Alcalay, E Giladi, A Pinhasov, S Furman, DE Brenneman NAP: a neuroprotective peptide. J Mol Neurosci 2002;19:167-70
3. I Gozes, AD Spier Peptides as drug targets in Alzheimer's disease. Drug Dev Res. 2002;56: 475–81
4. I Gozes, I Divinsky, I Pilzer, M Fridkin, DE Brenneman, AD Spier From VIP through ADNP to NAP: *A view of neuroprotection and cell division* J Mol Neurosci 2003;20:315-22
5. I Gozes, RA Steingart, AD Spier NAP mechanism of neuroprotection. J Mol Neurosci 2004;24:67-72
6. I Gozes, I Divinski The femtomolar-acting NAP interacts with microtubules: Novel aspects of astrocyte protection. J Alzheimers Dis. 2004;6(6 Suppl):S37-41
7. I Gozes. Neuroprotection. Encyclopedia of Neurosci,3rd addition,2004;http://www1.elsevier.com/homepage/sah/ens/
8. I Gozes, R. Zaltzman, J Hauser, DE Brenneman, E Shohami, JM Hill. The expression of activity-dependent neuroprotective protein (ADNP) is regulated by brain damage and treatment of mice with the ADNP derived peptide, NAP, reduces the severity of traumatic head injury. Curr Alzheimer Res. 2005;2:149-53

C. Research Support: Ongoing Research Support

October 2003-September 2007 Allon Therapeutics through Ramot (PI). Goals: Understanding NAP and pipeline peptide products toward neuroprotective drug development
October 2004- September 2008- The US-Israel Binational Science Foundation (PI), together with Drs. Peng Y. Loh and Dr. Joanna Hill. Understanding ADNP processing into smaller molecules.
October 2003-September 2007- The Israel Science Foundation (PI). ADNP in CNS development
October 2004- NIA contract. Ongoing contract for NAP toxicology (consultant)
March 2006 – Nofar – Governmental-Industrial Support (Cancer Research)

Completed Research Support (selected)

October 1999- September 2004 NIA contract N01-AG-9-2117 NAP toxicology (consultant)
October 1999-September 2003 The Israel Science Foundation (PI). NAP related peptides
1999 – 2002, The Institute for the Study of Aging (ISOA) (PI) Femtomolar-Acting Neuroprotective Peptides: Application for Inhibition of Neurodegenerative Disease; 2003 – 2005 (PI) NAP toward phase I clinical trials.
October 2000-September 2003 BSF (PI), together with Dr. DE Brenneman (NIH). Understanding ADNP

Volume 310, number 3, 205–210 FEBS 11589 October 1992

Hypothesis

Reversal of peptide backbone direction may result in the mirroring of protein structure

P. Guptasarma

Centre for Cellular and Molecular Biology, Hyderabad-500 007, AP, India

Received 1 June 1992; revised version received 14 August 1992

In linear polypeptides, inversion of amino acid chirality (all-L to all-D) achieves a mirroring of side chain positions and interactions in conformational space. A similar mirroring of side chain positions is independently achieved by a reversal of the direction of the peptide backbone (retro modification). Thus, while an all-D chain could be expected to adopt a perfect 'mirror image' of the three-dimensional structure of its parent all-L protein, the retro-all-L chain could be expected to adopt a topological equivalent of such a mirror image, through the symmetry transformations of side chain interactions. These notions, supported by sequence analyses, modelling studies, and evidence relating to the activity of 'retro–inverso' peptides, are extended towards the proposal, that the backbone reversed chain of a large globular protein might recognize the chiral opposite of the parent protein's substrate(s).

Retroprotein; Peptide backbone direction; Sequence analysis; Structure modelling; Chirality; Protein engineering

1. INTRODUCTION

The polypeptide chain of a natural protein may be called a normal-all-L chain; 'normal' defining the direction of peptide bonds along the chain, and 'all-L' defining the chirality of the amino acids used to build it. Using the condition that any transformation of the chain, by switching of amino acid direction or chirality (or both) must be uniformly applied to every residue in the chain, it is possible to think of three deviant forms of the normal-all-L polypeptide, shown in Fig. 1. These are the normal-all-D (inverso), retro-all-L (retro) and retro-all-D (retro-inverso) forms.

Attempts have been made to introduce retro- and inverso modifications of a few different kinds in small peptide hormones [1]. The results of studies conducted on peptides modified throughout the length of the chain, are as follows: normal-all-D [2–6] and retro-all-L [7,8] analogues generally do not possess biological activity. On the other hand, retro-all-D analogues have been found to possess biological activity [1,9–11] when their end groups are suitably modified [1]. The effect of the retro modification thus opposes the effect of chirality inversion, indicating that the two modifications achieve the same transformation with respect to the spatial positions of amino acid side chains. Each modification independently mirrors the side chain across the extended peptide backbone, as is obvious from a consideration of Fig. 1.

The nature of these structural relationships is discussed below in the form of a few 'Gedanken experiments', which consider various aspects of the consequences of these modifications on the structures of globular proteins. Briefly, these experiments lead to the following: (i) a new approach to understanding the topological equivalence between the retro-all-D form and the parent chain; (ii) a reiteration of the enantiomeric relationship between the normal-all-D form and the parent chain; and (iii) a derivation of the structural relationship between the retro-all-L form and the parent chain. This last relationship is shown to involve a topological mirroring of protein structure.

2. STRUCTURAL RELATIONSHIPS

2.1. *The retro-all-D polypeptide*

Let us imagine that it is possible to convert, instantaneously, every C=O atom pair in the peptide backbone of a compactly folded globular protein into an N–H atom pair, and vice versa. Would such a conversion force a change in the structure of the folded protein? It would seem that it would not, for the following reasons. The interchange would not redistribute any major charge centers. Nor would it be sterically disallowed, since, (i) the peptide bond is essentially planar in nature, (ii) the lengths of the C=O and N–H bonds (C=O ≈ 1.20 Å and N–H ≈ 1.03 Å) are not very differeni, (iii) the bond angle Cα–C=O in proteins (≈120°) is roughly

Correspondence address: P. Guptasarma, Centre for Cellular and Molecular Biology, Hyderabad-500 007, AP, India. Fax: (91) (842) 851 195.

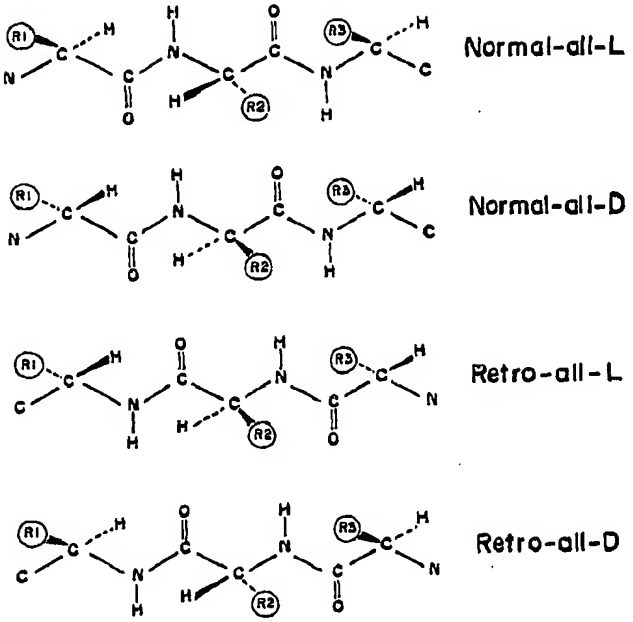

Fig. 1. The variants of the normal-all-L polypeptide obtained by inverting amino acid chirality (normal-all-D), reversing peptide backbone direction (retro-all-L), or both (retro-all-D). R1, R2 and R3 represent the side chains attached to α carbon atoms.

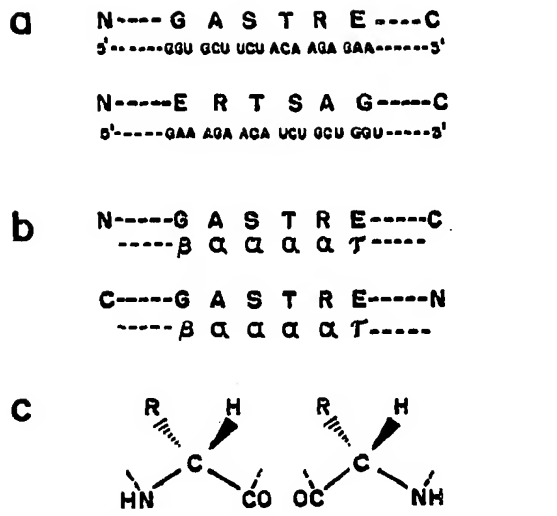

Fig. 2. (a) Corresponding segments of a protein (above) and its retro-all-L isomer (below) running in mutually reversed directions; nucleotide sequences are shown below the amino acids. (b) Schematic representation of hypothetical secondary structure prediction profiles (β, beta-structure, α, alpha-helix, τ, turn) of the two sequences. On lateral inversion of the representation of the retro isomer in (a), the predicted profiles are found to overlap. (c) Exchange of the C=O and N–H atom pairs of a residue in a folded protein, without modification of side chain position, amounts to an inversion of chirality. Note: the dashed lines in all three figures indicate the extension of the polypeptide chain on both sides of the chain.

equal to the bond angle Cα–N–H (≈115°), (iv) the van der Waals radii of the atoms involved (C = 1.65, N = 1.55, O = 1.50 and H = 1.20–1.45, Å) are comparable, and (v) the angle between the C–Cα and Cα–N bonds (≈110°) is not affected by the transformation. The transformation could, therefore, be expected not to disturb the protein's topology. Let us consider what it amounts to.

There is an obvious reversal of peptide backbone direction owing to the interconversion of the C=O and N–H atom pairs. Since the side chains are not moved from their positions, the reversal of backbone direction is effectively mediated through the exchanging of positions between the occupants of two of the four corners of the tetrahedron surrounding each α carbon atom in the chain (Fig. 2c). This amounts to an inversion of amino acid chirality, as well. The protein obtained is thus a retro-all-D molecule, which is topologically equivalent to the parent normal-all-L protein. Since the C–N interchange replaces the torsion angle C–N–Cα–C with N–C–Cα–N, the dihedral angle ϕ characterizing any residue in the retro-all-D protein would approximately equal the dihedral angle ψ associated with the same residue in the parent protein.

2.2. *The normal-all-D polypeptide*

In the case of short peptides, it is easy to see that the mirroring of side chain positions in conformational space due to the L-to-D conversion would result in symmetry transformations (mirroring) of side chain interactions as well. For a globular protein refolding from the denatured state, such a mirroring of interactions may be expected to make the molecule fold into a three-dimensional structure which would constitute a perfect mirror image of the native structure of the normal-all-L parent protein. The rotations about the N–Cα and Cα–C bonds necessary for such mirroring are sterically and energetically allowed; Ramachandran et al. [12] have suggested that the preferred conformational space of D-amino acid residues inclined to participate in the formation of regular secondary structures ($-\phi,-\psi$), is obtained by reflecting the conformational space of L-amino acid residues across the origin of the Ramachandran diagram. A right-handed alpha-helix in the parent protein would, therefore, be represented by a left-handed helix in the normal-all-D protein. Polypeptide chains made of D-amino acids might therefore adopt structures that would mirror their parent molecules so completely that they could recognize the chiral opposites of the substrates of the original peptides.

2.3. *The retro-all-L polypeptide*

Reversal of peptide bond direction achieves a mirroring of side chain positions in conformational space when it is not accompanied by inversion of amino acid chirality (Fig. 1). The resulting symmetry transformations of the side chain interactions could therefore cause

the retro-all-L chain to fold, as well, into a mirror image of the parent normal-all-L protein. This mirror image would, however, be only a topological equivalent of the structure adopted by the normal-all-D protein, since every C=O atom pair in the latter would be represented by an N–H atom pair in the former.

The dihedral angle ϕ, corresponding to any residue in the retro-all-L protein, would therefore represent the dihedral angles ψ, $-\psi$, and $-\phi$ respectively, in the normal-all-D, normal-all-L and retro-all-D proteins. It is noted parenthetically that a change of sign in these transformations indicates a mutual mirroring of side chain positions, while a change of dihedral angle indicates the replacement of the torsion angle C–N–Cα–C, by the torsion angle N–C–Cα–N, owing to the reversal of backbone direction. The relationship of the parent normal-all-L molecule to its three deviant forms is outlined below.

normal-all-D: ϕ corresponds to $-\phi$ of parent molecule (and ψ to $-\psi$) (I)

retro-all-L: $-\phi$ corresponds to ψ of parent molecule (and $-\psi$ to ϕ)? (II)

retro-all-D: ϕ corresponds to ψ of parent molecule (and ψ to ϕ) (III)

It may be noted that the transformations described by expressions I and II, add up to that described by expression III, as expected.

3. MODELLING THE RETRO-ALL-L STRUCTURE

The following computational experiment was done to test the validity of the proposed mirror imaging principle. The pair of mutually retro-running hexapeptides, H_2N–GASTRE–COOH (comprising residues 56–61 of the hypervariable L2 loop in the light chain of the phosphorylcholine binding antibody, McPC 603 [13]), and its retro-peptide, H_2N–ERTSAG–COOH, were constructed in an extended conformation on the Desktop Molecular Modeller (DTMM, Version 1.0, (C) Oxford University Press, 1989) software, and put through an energy minimization routine using the derivative method developed by Vinter et al. [14], on an IBM PC/AT 386 equipped with an 80387 coprocessor and a 4 MB RAM, running at a clock speed of 25 MHz. Identical side chain conformations were used for corresponding amino acids in the two chains. Energy minimization was carried out for 1,000 cycles of iteration during which simultaneous minimization of bond length energy, bond angle energy, torsion angle energy and van der Waals interaction energy was carried out.

Fig. 3 shows the starting and final conformations of

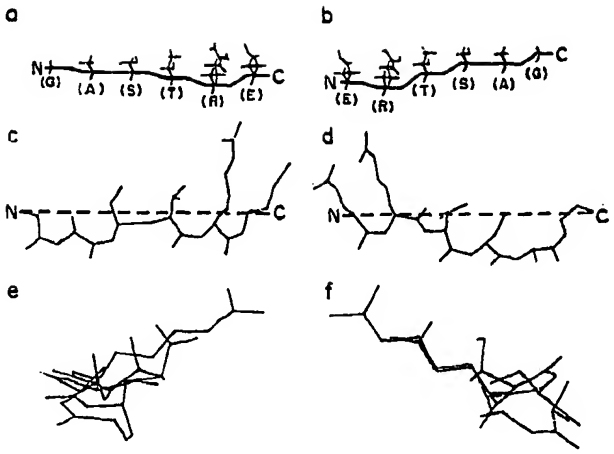

Fig. 3. (a and b) The two chains, H_2N–GASTRE–COOH and H_2N–ERTSAG–COOH, as constructed on DTMM, prior to minimization. The energies of the molecules are 27,984 and 20,588.3 kJ/mol, respectively. (c and d) The corresponding structures obtained after 1,000 iterations. The energies of the molecules at this stage are 3,729.28 and 3,901.49 kJ/mol, respectively. The dotted line joins the carbon of the C-terminal carboxyl group to the nitrogen of the N-terminal amino group. Of the atoms at the termini, only N and C are shown here. (e) View of (c), looking down from N to C. (f) View of (d) looking down from C to N. The amino acid G (Gly) is on top in both cases.

the two molecules, while Fig. 4 shows a comparative view of their peptide backbones. The structures in Fig. 3 tend to mirror each other topologically in a manner that could allow them to recognize chirally opposite substrates. At some α carbons, the corresponding dihedral angles ϕ and ψ, can already be seen to be tending towards equal and opposite values at the end of 1,000 iterations. Emphasis here is laid not on the accuracy or feasibility of structure prediction by energy minimization methods, but on the fact that the same considerations during energy minimization (applied for the same number of iterations, to two starting structures made with identical side chain conformations, and no bias in backbone conformation) resulted in such structures.

4. ANALYZING RETRO-ALL-L SEQUENCES

What about large proteins with significant amounts of secondary structure? Would regions of the retroprotein, corresponding to helix- or sheet-forming regions in the parent protein, be equally disposed to forming regular structures? The transformations described in expressions I and II show that residues participating in right-handed alpha-helices in the normal-all-L chain are likely to form left-handed helices in both the normal-all-D and retro-all-L chains, since the ϕ,ψ pairs obtained by both transformations fall in the 'left-handed helix' region of the Ramachandran diagram. Few would contest the suggestion that a right-handed helix in a normal-all-L protein would be replaced by a left-handed helix in its normal-all-D counterpart. Considering that most

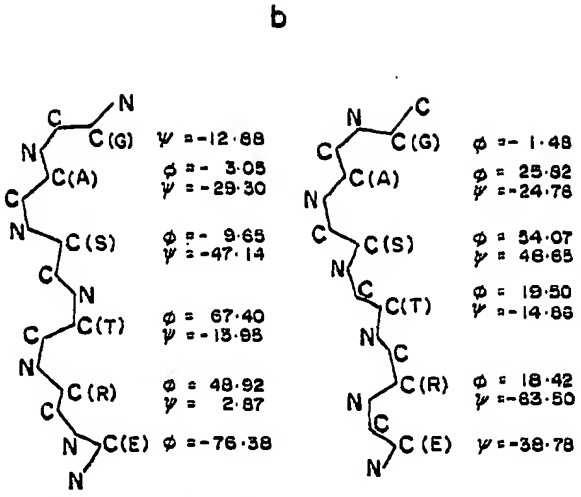

Fig. 4. (a) Peptide backbone of the molecule shown in Fig. 3c, rotated clockwise by about 90°. (b) Peptide backbone of the molecule shown in Fig. 3d, rotated anti-clockwise by about 90° and then rotated by 180° about the C–N axis.

known all-L proteins possess only right-handed helices, however, the plausibility of the second contention, namely that a similar replacement would occur in the retro-all-L protein as well, needs to be examined. Can an all-L protein contain left-handed α-helices? Or, for that matter, can an all-D protein contain right-handed helices, as is suggested by our earlier consideration that the retro-all-D protein would be equivalent to the normal-all-L protein?

That the left-handed helical conformation is not disallowed in all-L chains per se is evident from the fact that left-handed helical residues are not unknown among proteins crystallized to date [15,16]. While glycine adopts right- and left-handed helical conformations with roughly equal frequencies, a large number of non-glycine residues, too, are found in the left-handed helical conformation [16], indicating that L-amino acids are not sterically disallowed from adopting such a conformation, in an otherwise all-L context. Weaver et al. [17] found that glycines and non-glycines occurring in left-handed helical conformation in loop regions, or at the termini of helices, have very similar backbone conformational energies (within 0.5 kcal/mol of each other). This indicates that, energetically speaking, left-handed conformations of this kind are not prohibited. But then, how does the existence of the occasional left-handed alpha-helical residue in natural all-L proteins relate to the larger question of the formation of a left-handed alpha-helix? Obviously, the formation of such a structure would require that a stretch of contiguously placed residues in a chain be similarly disposed to adopting a left-handed helical conformation. Would such a stretch be allowed to form a left-handed helix?

Ramachandran et al. [12] point out that both the right- and left-handed alpha-helical forms lie outside the 'normally allowed' regions of the Ramachandran diagram but within the 'outer limits' of the sterically allowed regions. In L-amino acid chains, the perfect right-handed alpha-helical form ($\phi = -50$, $\psi = -50$) is somewhat more stable than the perfect left-handed alpha-helical form ($\phi = 50$, $\psi = 50$), while the converse is true for D-amino acid chains. Helices in proteins, however, rarely conform to such standards; the average helix in a natural protein is a substantially distorted version of the perfect right-handed alpha-helix [15]. Thus, while it is true that right-handed helices of L-amino acids are likely to tolerate much more distortion than left-handed helices [12], since they occupy a larger region of the Ramachandran map, some sequences of L-amino acids could find themselves having to choose between: (i) adopting a very distorted (unstable) right-handed alpha-helical structure; and (ii) adopting a not-so-distorted (slightly more stable) left-handed alpha-helical structure. In such a situation, some sequences might very well adopt continuous left-handed helices.

Perhaps the reason that we do not see such structures in natural proteins is a consequence of the fact that sequences inclined to form such structures have not been chosen by evolution, or have otherwise gone undetected. I suggest that the retro-all-L sequences of natural proteins constitute such sequences, and that, therefore, they have a very fair chance of adopting left-handed alpha-helices.

How does the extended (beta) conformation respond to the $\phi = -\psi$, $\psi = -\phi$ transformation achieved by the retro-all-L modification? This question is most easily addressed by taking the coordinates of any point from within the region defining the beta-structure in the Ramachandran diagram, and putting it through the above transformation to see where it lies after the transformation. Such an exercise shows that, for every such point, the coordinates of the transformed point continue to lie within the same region of the map; for instance (−150,100) becomes (−100,150). Thus, the retro-all-L modification would appear to conserve extended beta-structures.

How do available routines for protein sequence analysis respond to the suggestion that secondary structural elements are conserved through backbone reversal? Before such a question is addressed, of course, it is necessary to realize the two predictable consequences of asking such a question: (i) no available program could be expected to predict the occurrence of a left-handed alpha-helix; and (ii) some of these programs use an empirically derived set of structure forming probabilities, to examine the probability of formation of a particular secondary structure by an amino acid, in the context of its immediate neighbourhood. Since the neighbourhood of any residue in a normal-all-L protein happens to be equivalent to that of its corresponding resi-

due in the retro-all-L protein, the outputs of such programs cannot be influenced substantially by the direction of the chain. Thus, the predictions for a natural protein and its retro-protein, represented graphically as plots of calculated amino acid characteristics (Y-axis) vs. amino acid sequence (X-axis), may be expected to mirror each other across the Y-axis (or be superimposable upon lateral inversion of either profile). As is described below, this is indeed what happens.

A number of protein sequences were reversed and analyzed on the sequence analysis software PC/Gene (Version 6.50, (C) Amos Bairoch, University of Switzerland, Geneva). The software was used to carry out secondary structural (and other) analyses, using the routines ANTIGEN (antigenicity of fragments), SOAP (hydropathy profile), GGBSM, GARNIER, NOVOTNY, BETATURN (secondary structure prediction), RADIALOC (prediction of radial locations in globular proteins) and FLEXPRO (prediction of chain flexibility). In some secondary structure prediction routines, i.e. those which output very different predictions for the normal-all-L and retro-all-L sequences when default settings of user-defined parameters (e.g. percentages of secondary structures etc.) are used, these parameters were adjusted to predict a close match to the known secondary structure of a protein before the routine was applied to analyzing its retro-protein.

As expected, the predictions for mutually reversed chains mirrored each other (Fig. 2b illustrates this point schematically). Thus, the regions predicted to form helices, extended sheets, and even beta-turns, comprise the same set of residues in two proteins with mutually reversed chain directions. For instance, the residues 4–7 and 40–43 in the 46 residue plant toxin, crambin (sequence obtained from [18]), which have the highest predicted potential for beta-turn formation, overlap (three residues out of four) with regions 41–44 and 5–8, which have the highest corresponding potential in the retro-crambin sequence obtained by reversing the crambin sequence. The shift occurs presumably to accommodate the residue, proline, in the second position of the turn. Similarly, the retro-sequence of the protein, felix (a de novo designed four helix bundle) turns out to have the same groups of residues forming predicted helices and beta-turn-containing loops, as its parent protein (sequence from [19]).

Although these outcomes appear to support the contentions made in this paper, their significance may be called into question, since the methods used for these predictions are somewhat insensitive to chain direction. However, considering that these programs use a knowledge-based approach to predict the secondary structures of all-L chains with a modest degree of success [20], the prediction of beta-structures, in line with the expectations outlined earlier, may be taken to support the notion that a beta-structure remains a beta-structure even upon retro-all-L modification. Alternatively, these results probably only bring to light a serious lacuna in current secondary structure prediction methods. Certainly, the prediction of helical stretches in corresponding segments of the two proteins arises from the identification and propagation of helix-nucleating regions in a direction-independent manner.

5. PERSPECTIVES

Retroproteins are likely to adopt three-dimensional structures which are topological mirror images of the native structures of the parent proteins. They could thus recognize and modify the chiral opposite(s) of the parent protein's substrate(s). While such a function would, in all likelihood, be performed just as well by normal-all-D molecules, these are not easily synthesized. Retroproteins, on the other hand, are very easily made in living systems (Fig. 2a) through modern recombinant DNA technology. Such molecules could find applications in the introduction of new metabolic pathways into living systems, e.g. enzymes designed to recognize and use L-glucose could enhance the nutritional adaptability of microorganisms. Others could be used to produce unnatural isomers of biological molecules inside living systems, or distinguish between chirally unselected products of chemical reactions.

Acknowledgements: For insightful discussions and help with the preparation and editing of this manuscript, I am deeply indebted to Prof. D. Balasubramanian, CCMB, Hyderabad. I am also indebted to Manni Guptasarma and Dipankar Manna, CCMB, for helpful discussions, and to Sushil Chandani for comments on the manuscript. I thank Dr. Matti Saraste, Editor, FEBS Letters, for bringing independently to my notice, an article by Kent and colleagues ([1992] Science 256, 1445), which appeared during the refereeing of this manuscript. This article shows that the normal-all-D HIV protease does actually fold into a structural mirror image of its parent protein to recognize the chiral opposite of its substrate, as proposed in this paper.

REFERENCES

[1] Goodman, M. and Chorev, M. (1979) Acc. Chem. Res. 12, 1.
[2] Schroeder, E., Lubke, K. and Hempel, R. (1965) Experientia 21, 70.
[3] Stewart, J.M. and Wooley, D.W. (1965) Nature 206, 619.
[4] Flouret, G. and du Vigneaud, V. (1965) J. Am. Chem. Soc. 87, 3775.
[5] Vogler, K., Studer, R.O., Lergier, W. and Lanz, P. (1965) Helv. Chim. Acta 48, 1407.
[6] Morley, J., Tracy, H.J. and Gregory, R.A. (1965) Nature 207, 1356.
[7] Bodanszky, M., Ondetti, M.A. and Sheehan, J.T. (1963) Ann. NY Acad. Sci. 104, 24.
[8] Vogler, K., Lanz, P. and Lergier, W. (1962) Helv. Chim. Acta 45, 561.
[9] Chorev, M., Willson, G.C. and Goodman, M. (1977) J. Am. Chem. Soc. 99, 8075.
[10] Shemyakin, M.M., Ovchinnikov, Yu.A. and Ivanov, V.T. (1969) Angew. Chem., Int. Ed. Engl. 8, 492.
[11] Goissis, G., Nonalhetas, V.L.A. and Paiva, A.C.M. (1976) J. Med. Chem. 19, 1287.

[12] Ramachandran, G.N., Ramakrishnan, C. and Sasisekharan, V. (1963) J. Mol. Biol. 7, 95.
[13] Satow, Y., Cohen, G.H., Padlan, E.A. and Davies, D.R. (1986) J. Mol. Biol. 190, 593.
[14] Vinter, J.G., Davis, A. and Saunders, M.R. (1987) J. Comp. Aided Mol. Design 1, 31.
[15] Presta, L.G. and Rose, G.D. (1988) Science 240, 1632.
[16] Nicholson, H., Solderlind, E., Tronud, D.E. and Matthews, B.W. (1989) J. Mol. Biol. 210, 181.
[17] Weaver, L.H., Tronud, D.E., Nicholson, H. and Matthews, B.W. (1990) Curr. Sci. 51, 833.
[18] Teeter, M.M., Mazer, J.A. and L'Italien, J.J. (1981) Biochemistry 20, 5427.
[19] Hecht, M.H., Richardson, J.S., Richardson, D.C. and Ogden, R.C. (1990) Science 249, 884.
[20] Garnier, J. (1990) Biochimie 72, 513.